

# Biochemical Markers of Possible Immunodepression in Military Training in Harsh Environments

Linda M. Castell, MSc\*; C. Douglas Thake, PhD†; Wayne Ensign, PhD‡

**ABSTRACT** Prolonged, exhaustive exercise frequently leads to an increased incidence of upper respiratory tract illness (URTI) which is linked to transient immunodepression. We investigated potential biochemical markers of stress and fatigue, and URTI symptoms as a surrogate of immunodepression, in US Marines undergoing intensive winter training at altitude. Selected plasma amino acids and leptin (p[Lep]) were measured as possible markers of fatigue and immunodepression, together with nonesterified fatty acids (p[NEFA]) and total antioxidant capacity (p[TAC]). Changes were observed in plasma free tryptophan (p[FT]), p[Gln], p[Lep], p[NEFA], p[TAC] but not branched chain amino acids (p[BCAA]). p[FT] decreased markedly. Resting p[Gln] decreased overall after one month at altitude. p[Gln] routinely decreases 1–2 hrs after prolonged exercise. Importantly, we observed early morning decreases in p[Gln], suggesting a cumulative effect of prolonged activity, stress, and fatigue. Concomitantly, individuals with highest illness scores had the greatest p[Gln] decrease: low p[Gln] may therefore be associated with a diminished stress tolerance.

## INTRODUCTION

The degree of stress associated with military operations is considerable for the individual. Physical and psychological demands can be exacerbated by sleep deprivation, by inadequate caloric intake, and by a disruption of normal sleep/rest/eating cycles. Changes in environmental conditions and the individual's adaptive response to exposure all contribute to the degree of stress-related impairment of immune function and consequent susceptibility to opportunistic infectious agents. This study aimed to investigate potential biochemical markers as indicators of stress and fatigue and evaluate their relation to upper respiratory tract infection (URTI) in a cohort of U.S. marines undergoing military training at altitude during winter.

Few studies to date have evaluated the long-term consequences of military training on immunological function in individual military personnel. Kramer et al.<sup>1</sup> evaluated T-lymphocyte responses during an army ranger course but did not evaluate biochemical changes that might be related to immune function or their relation to the occurrence of URTI. Their study clearly demonstrated immunological changes during ranger training. However, they had no follow-up in their study so it is unclear how rapidly these alterations returned to normal levels. Whitham et al.<sup>2</sup> studied paratroopers for 19 weeks, training at sea level. Their main findings were an increase in URTI in weeks 2 and 3 and a progressive decrease in salivary flow rate during the study, which might have led to hypohydration. The present study links certain biochemical markers to the severity and operational impact

of subsequent illnesses that may be due to immunodepression and to recovery from winter field training exercises at altitude.

Markers of free radical formation increase during strenuous exercise in athletes and nonathletes alike. In most cases, these markers return to normal levels after cessation of physical activity.<sup>3</sup> However, an important factor is the time available for rest and recuperation. In military personnel undergoing training in harsh conditions, the continuous exposure to different types of stress for prolonged periods of time, is likely to lead to insufficient recovery. Thus, the exposure to oxidative stress is likely to be far greater among military personnel than those in nonmilitary occupations or athletes undertaking regular exercise.

## Exercise, Glutamine, and Immunodepression

Glutamine provides nitrogen for purine and pyrimidine nucleotide synthesis, for synthesis of new DNA and RNA during lymphocyte proliferation and for mRNA synthesis and DNA repair in macrophages. Glutamine can become conditionally essential when cells are stimulated to increase their production during trauma, infection, burns,<sup>4,5</sup> and tissue damage. In *in vitro* studies, Parry-Billings et al.<sup>5</sup> demonstrated that decreasing the concentration of glutamine in culture medium, despite the presence of all other nutrients, both reduced the proliferative ability of lymphocytes and slowed their response time to a mitogenic stimulus.

Previous studies from our group observed decreases in the p[Gln] (up to 25% within 2 hr after a marathon), transient depression of immune function, and an increase in the incidence of infections after prolonged, exhaustive exercise.<sup>6,7</sup> Low resting levels of p[Gln] have been observed in athletes with unexplained underperformance syndrome and fatigue.<sup>8,9</sup> In a pilot study we made similar observations in marines after a short sojourn at altitude. However, the study was not specifically designed to address the present issues (L. Castell and

\*University of Oxford, Green Templeton College, Woodstock Road, Oxford OX2 6HG, U.K.

†Coventry University, Department of Biomolecular & Sport Sciences, Faculty of Health & Life Sciences, James Starley Building, Priory Street, Coventry CV1 5FB, U.K.

‡SPAWARSSYSCEN 71730, 49590 Lassing Road, Room A-339, San Diego, CA 92152-6343.

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE <b>MAR 2010</b>		2. REPORT TYPE		3. DATES COVERED <b>00-00-2010 to 00-00-2010</b>	
4. TITLE AND SUBTITLE <b>Biochemical Markers of Possible Immunodepression in Military Training in Harsh Environments</b>				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>SPAWAR Systems Center, 49590 Lassing Road. Room A-339, San Diego, CA, 92152-6343</b>				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release; distribution unlimited</b>					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT <b>see report</b>					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT <b>Same as Report (SAR)</b>	18. NUMBER OF PAGES <b>9</b>	19a. NAME OF RESPONSIBLE PERSON
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>			

W. Ensign, unpublished data). It has not yet been established whether the plasma glutamine decrease is a result of altitude per se or is an effect of the intensive exercise undertaken at altitude.

After strenuous exercise, there is evidence of immunodepression and an increase in the incidence of illness, particularly URTI.<sup>10,11</sup> Evidence for exercise-induced immunodepression is provided by the reduction of circulating lymphocyte numbers and function to below baseline levels in many athletes early in the recovery period after prolonged, exhaustive exercise. Natural killer cells are markedly decreased 16 hr after a marathon race<sup>29</sup> and do not return to normal for up to 48 hr. Neutrophil function decreases in cyclists undertaking prolonged bouts of exercise at 55% and 80%,<sup>27</sup> and in endurance athletes immediately after  $\text{VO}_{2\text{max}}$  tests.<sup>28</sup> The general consensus among exercise and stress researchers is that stress-induced immunodepression is associated with the risk, or cause of, URTI. For examples, see<sup>41,42</sup>. Thus the incidence of URTI symptoms was used as a surrogate for immunodepression in this study.

In a double-blind study, glutamine supplementation vs. placebo was given to marathon runners ( $n = 37$ ) after a race: there was a significant reduction in their self-reported incidence of illness, in particular URTI, with 81% in the glutamine group being free of infections vs. 49% in the placebo group.<sup>6</sup> Exogenous provision of glutamine has also had a beneficial effect in several different clinical situations, including major surgery.<sup>12</sup> However, it is still not clear what role glutamine supplementation has in terms of depressed immune function in exercise.

Circulating numbers of neutrophils returned more rapidly to normal levels 16 hr after a race in marathon runners who had received glutamine supplementation, compared with a placebo.<sup>29</sup> Krzywkowski et al.<sup>30</sup> found less neutrocytosis in athletes receiving sufficient glutamine vs. placebo to maintain plasma levels during strenuous exercise.

A significant decrease in production of the neutrophil chemoattractant, IL-8, occurred in marathon runners given glutamine vs. placebo after a race (see<sup>7</sup>). A similar effect on plasma IL-8 was observed in a study on glutamine-fed patients with acute pancreatitis.<sup>31</sup> Provision of exogenous glutamine may thus lead to enhanced function of neutrophils and a decrease in the requirement for IL-8 secretion to attract more neutrophils to the site of tissue damage.

### Central Fatigue and Amino Acids

The plasma concentration ratio of free tryptophan to branched chain amino acids ( $[\text{pFT}/\text{p(BCAA)}]$ ) was measured in this study as a potential biochemical marker for central fatigue.<sup>13,14</sup> Tryptophan is a precursor for 5-hydroxytryptamine (5-HT): uniquely it binds to albumin in the blood, for which it competes with free fatty acids (NEFA). Unbound (free) tryptophan (FT) competes with BCAA for the same port of entry into the brain across the blood-brain barrier. The  $[\text{pFT}]$  is usually increased in humans after prolonged exhaustive exercise.<sup>14</sup>

This may lead to an increase in the rate of synthesis in 5-HT, which is involved in sleep and fatigue. Central fatigue emanates from the brain as opposed to peripheral fatigue, which emanates from muscle.

### Leptin

Leptin, originally known as the "obesity hormone,"<sup>15</sup> has a role in the immune system.<sup>16</sup> Caldefie-Chez et al.<sup>17</sup> suggested that the leptin receptor may influence neutrophil oxidative capacity. The relationship between sleep and leptin levels<sup>18</sup> may be important in immune function. A significant decrease (24%) in plasma leptin was observed in most subjects after one night's sleep deprivation.<sup>19</sup> It is hypothesized that leptin will be affected both by altered sleep patterns and strenuous exercise in winter training at altitude.

The main objectives of the present study were to monitor  $[\text{pGln}]$  as a potential marker of susceptibility to URTI symptoms consistent with immunodepression, together with plasma leptin ( $[\text{pLep}]$ ); and the plasma concentrations of free tryptophan ( $[\text{pFT}]$ ), BCAA ( $[\text{p(BCAA)}]$ ), and NEFA ( $[\text{p(NEFA)}]$ ) as possible indicators of stress and central fatigue. An additional objective was to determine whether these parameters are linked to each other and/or to oxidative stress: they were also compared with the incidence of URTI symptoms. In addition, the effect of normobaric hypoxia at rest and exercise on  $[\text{pGln}]$  was compared with normoxia in a laboratory-based study.

### METHODS

Ethical permission was obtained for this study from the Naval Health Research Center's Institutional Review Board in accordance with Office of the Chief of Naval Operations (OPNAV) instructions. Sixty-one male combat infantry marines from a single battalion gave informed consent before undergoing a 6-month longitudinal assessment period (January–June 2001) while training in harsh environments. All participants were given a physical examination by the battalion surgeon and deemed healthy for winter operations. None of the participants was taking prescription medication. Further details of characteristics and military activities have been published elsewhere.<sup>40</sup> Immunological and disease-related outcomes were evaluated in each marine via six blood samples collected over 6 months. Functional and population measurements were made on some immune cells (data reported elsewhere). Plasma amino acids, and markers of oxidative stress were measured. Symptoms of disease, injury, and infections were monitored on a weekly basis in each marine by the platoon corporals using a symptom survey questionnaire (Fig. 1) during the assessment period.

### Experimental Design

The overall study design was as follows: Sixty-one marines from a single combat battalion based at Twentynine Palms, California (~1,500 ft) were recruited. Each individual was

**Marine Corps Immune Study:**  
(Symptoms Questionnaire)

Date: \_\_\_\_\_ Subject No. \_\_\_\_\_ Platoon: \_\_\_\_\_

**Within the last month have you had a fever blister or cold sore?**

No  
Yes  $\Rightarrow$  About how often: \_\_\_\_\_

**Do you use tobacco**

No  
Yes  $\Rightarrow$  Smoke or Chew. About how much \_\_\_\_\_

**Allergic Reaction:**

Itching/Watery eyes .....	1.	2.	3.	4.	5.
Scratchy Throat .....	1.	2.	3.	4.	5.
Sneezing .....	1.	2.	3.	4.	5.
Wheezing .....	1.	2.	3.	4.	5.

**Numerical Codes:**

1  $\Rightarrow$  Symptoms absent  
2  $\Rightarrow$  Symptoms present – no treatment  
3  $\Rightarrow$  Moderate – treatment sought  
4  $\Rightarrow$  Moderately severe – limited duty  
5  $\Rightarrow$  Severe – removal from duty

**Upper Respiratory Illness**

Dry Cough .....	1.	2.	3.	4.	5.
Productive Cough .....	1.	2.	3.	4.	5.
Sore Throat .....	1.	2.	3.	4.	5.
Hoarseness .....	1.	2.	3.	4.	5.
Stuffed-Up Nose .....	1.	2.	3.	4.	5.
Sinus Pain .....	1.	2.	3.	4.	5.
Fever .....	1.	2.	3.	4.	5.

**Musculoskeletal Injury:**

Aches/Joint Pain .....	1.	2.	3.	4.	5.
Soreness/Stiffness .....	1.	2.	3.	4.	5.
Cramps .....	1.	2.	3.	4.	5.
Strains/Sprains .....	1.	2.	3.	4.	5.

**Gastrointestinal Disorders:**

Upset Stomach .....	1.	2.	3.	4.	5.
Vomiting .....	1.	2.	3.	4.	5.
Diarrhea .....	1.	2.	3.	4.	5.

**FIGURE 1.** Marine Corps Immune Study: (Symptoms Questionnaire).

assessed for immunological function and overall health status over several months (January–June 2001). During this period, the marines were deployed to the Marine Corps Mountain Warfare Training Center (MUTC) at Bridgeport, California. Three individuals withdrew from the study at this point. Base camp was at 7,000 ft and the marines undertook field-training exercises (FTX) between 8,000 and 10,000 ft with occasional sojourns at 12,000 ft for 1 month at altitude in winter. Details of FTX have been outlined previously.<sup>40</sup>

Six blood samples were obtained, after overnight fasting, by qualified phlebotomists, from each marine volunteer between 5:00 a.m. and 7:00 a.m. over the course of the longitudinal assessment period at: (1) baseline (January), (2) within 24 hr of arrival at 7,000-ft base camp (FTX1) (mid February), (3) midway through FTX (FTX1b) (first week of March), (4) end of FTX (FTX2) (mid March), (5) 37 days after (Dsrt1) (mid April), and (6) 98 days after returning to desert (Dsrt2) (mid June).

**Experimental Methods**

After each collection, blood samples were centrifuged on site, serum/plasma was aliquotted and immediately stored frozen at  $-80^{\circ}\text{C}$  until shipped on dry ice to the United Kingdom where the amino acid, nonesterified fatty acids, leptin, and total antioxidant capacity (TAC) assays were undertaken.

**Plasma Assays**

Enzymatic assays were used to measure the plasma concentrations of glutamine<sup>20</sup> and BCAA.<sup>21</sup> Tryptophan was measured via high-performance liquid chromatography (HPLC), using a system dedicated to free and total tryptophan measurement. Plasma NEFA were measured with a Wako C enzymatic kit ( $\alpha$  Laboratories, United Kingdom). Plasma leptin was measured using a monoclonal antibody sandwich ELISA kit (R&D).

Plasma total antioxidant capacity was measured with the ABEL peroxyntirite TAC assay (Knight Scientific Ltd, Plymouth, UK) in which plasma antioxidants compete with the light-emitting protein Pholasin for peroxyntirite.<sup>43</sup> Total antioxidant capacity was presented as a vitamin E analog equivalent.

**Incidence of Illness**

Weekly records documenting symptoms related to disease, injury, and/or infection were collected from each of the participants during the 6-month assessment period using a standard form (Fig. 1). The platoon corporals underwent a training period before the study to ensure consistency of symptom scoring. Seven symptoms were evaluated associated with URTI: dry cough, productive cough, sore throat, hoarseness, stuffed-up nose, sinus pain, and fever. Each symptom was scored on a 5-point scale in which a score of 1 indicated no symptoms present and a score of 5 was given for symptoms severe enough to require removal from duty. The symptoms were then summated to yield a URTI symptom severity score. For the sake of brevity and consistency, a value of 7 was subtracted from the final value so that a numerical value of 0 represented the absence of any symptoms and a value of 1 indicated symptoms but untreated. While administering the symptom questionnaire, each participant was asked whether they had experienced a fever blister or cold sore within the last 4 weeks. It was assumed that the appearance of fever blisters and/or cold sores was caused by *Herpes simplex* virus HSV type I. Participants were also asked about allergic reactions.

**Hypoxia vs. Normoxia Study**

A balanced cross-over design was used in all the laboratory based hypoxia studies on healthy volunteers, not marines, for which separate ethical permission was obtained from the Coventry University Ethics Committee. All individuals undertook all procedures in random order, with a suitable recovery period in between. The protocol details were as follows:

- (i) Resting males ( $n = 16$ ): normoxia ( $N \gg F_{I}O_2 = 0.209$ ); hypoxia ( $H \gg F_{I}O_2 = 0.122$ , equivalent to ambient at  $\approx 4,000$  m) breathed between 0 and 240 min.
- (ii) Submaximal exercise ( $n = 7$ ): normoxia,  $N \gg F_{I}O_2 = 0.209$ ; hypoxia,  $H \gg F_{I}O_2 = 0.14$ , equivalent to ambient altitude at  $\approx 3,000$  m breathed 10 min before, during, and for 180 min after 10-min cycle exercise at  $80\% \text{VO}_{2\text{peak}}$ .
- (iii) Maximal exercise ( $n = 4$ ): normoxia,  $N \gg F_{I}O_2 = 0.209$ ; hypoxia,  $H \gg F_{I}O_2 = 0.14$ , equivalent to ambient at

»3,000 m breathed 10 min before, during, and for 180 min after incremental cycle exercise to volitional exhaustion. p[Gln] and p[NEFA] were measured.

#### Statistical Analysis

Repeated measures analysis of variance (ANOVA) was used, based on one repeated measures factor (collection periods) with no grouping variable. This analysis is similar to a single-factor analysis of variance with the degrees of freedom and assumptions adjusted to take into consideration the repeated measures aspect of the design. When statistical significance was observed for the global hypothesis (equality of means), multiple comparisons among means used the Neuman Keul's multiple comparison procedure and the paired Student *t*-test. All comparisons were made relative to the baseline values.

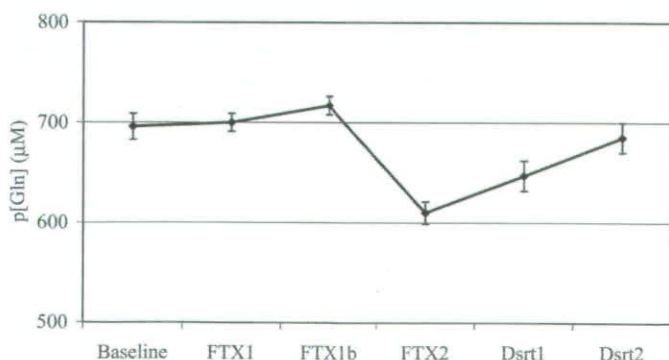
## RESULTS

### Symptoms Related to Upper Respiratory Tract Infections

There was a clear indication of URTI symptoms characteristic of immunodepression in the marines after 1 month's training at altitude in winter. At FTX2, 78% (45/58) developed URTI severe enough to require medication and/or limited duty, and 21% (12/58) developed fever blisters consistent with an active Herpes infection.

#### Glutamine

There was a significant decrease (12.4%,  $p < 0.001$ ) in the mean resting p[Gln] in the marines after 4 weeks intensive FTX (Fig. 2). This occurred concomitantly with a high incidence of URTI symptoms. In most cases, in those individuals with the highest illness scores, p[Gln] was decreased more (Table I). Interestingly, no differences were observed between p[Gln] responses to either moderate or intense laboratory-based cycling in normobaric hypoxia compared to those in normoxia. However, because of an increase in normoxia only, p[Gln] was lower than that in normoxia after breathing gas equivalent to 4,000 m for 120 min at rest ( $p < 0.052$ ).



**FIGURE 2.** Plasma glutamine (p[Gln]) in 58 marines training at altitude in winter (FTX) and desert. Compared with baseline (in desert) 14% and 8% decreases ( $p < 0.001$ ) occurred at FTX2 and Dsrt 1. Decrease at Dsrt 2 was not significant.

**TABLE I.** Changes in p[Gln] (Baseline vs. End of FTX) in Marines with Elevated URTI Symptoms Scores

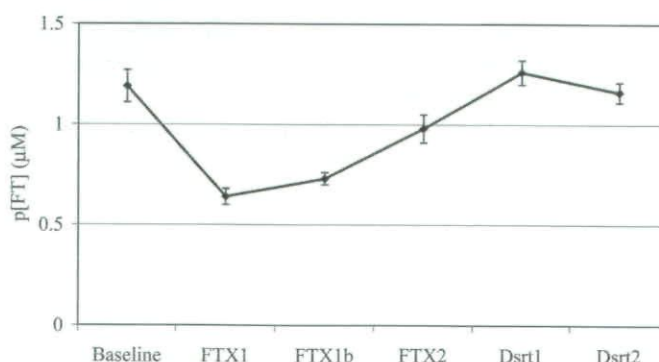
	Baseline	End of FTX	Subject No.	% Change
URT I Symptoms Score	1	5		
p[Gln]	620	506	2	-18
URT I Symptoms Score	0	5		
p[Gln]	591	652	15	9
URT I Symptoms Score	0	5		
p[Gln]	575	535	19	-7
URT I Symptoms Score	1	3		
p[Gln]	605	537	20	-11
URT I Symptoms Score	2	7		
p[Gln]	927	596	32	-36
URT I Symptoms Score	0	8		
p[Gln]	803	748	33	-7
URT I Symptoms Score	0	4		
p[Gln]	631	529	34	-16
URT I Symptoms Score	0	5		
p[Gln]	710	524	36	-26
URT I Symptoms Score	0	5		
p[Gln]	928	739	39	-20
URT I Symptoms Score	0	5		
p[Gln]	750	708	45	-6
URT I Symptoms Score	0	3		
p[Gln]	731	679	49	-7
URT I Symptoms Score	1	5		
p[Gln]	729	595	52	-18
URT I Symptoms Score	0	5		
p[Gln]	849	579	54	-32
URT I Symptoms Score	0	4		
p[Gln]	691	638	59	-8

#### Tryptophan

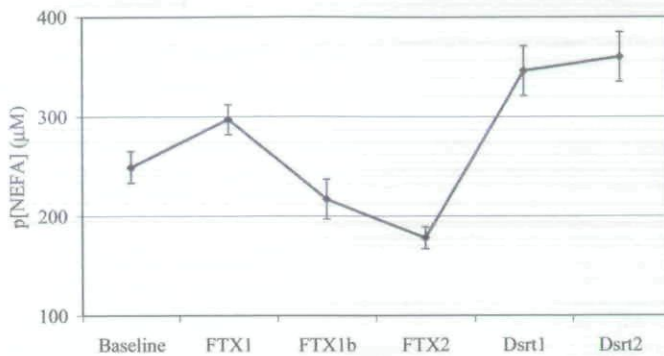
There was a significant decrease (46%,  $p < 0.001$ ) in p[FT] in all samples after arrival at altitude (Fig. 3). This was maintained during the mountain winter training phase and was concomitant with the start of the increased URTI. p[FT] returned to baseline levels at Dsrt2.

#### Branched Chain Amino Acids

No significant changes at any of the time points were observed (data not shown).



**FIGURE 3.** Plasma free tryptophan (p[FT]) in 58 marines training at altitude in winter (FTX) and desert. cf. baseline (in desert). FTX1: 46% decrease ( $p < 0.001$ ); FTX1b: 39% decrease ( $p < 0.001$ ); FTX2: 18% decrease ( $p < 0.05$ ).



**FIGURE 4.** Plasma nonesterified fatty acids (p[NEFA]) in 58 marines training at altitude in winter (FTX) and desert. Cf. baseline (in desert) FTX1b: 32% decrease ( $p < 0.01$  cf. FTX1); FTX2: 29% decrease ( $p < 0.001$ ); Dsrt1: 28% increase ( $p < 0.01$ ); Dsrt2: 31% increase ( $p < 0.001$ ).

#### Nonesterified Fatty Acids

An increase (10%,  $p < 0.05$ ) in the concentration of plasma nonesterified fatty acids (p[NEFA]) was observed on arrival at altitude (Fig. 4), which coincided with the decrease in p[FT] (Fig. 3). p[NEFA] subsequently decreased to below baseline halfway through and at the end of the FTX session. In another laboratory-based study (D. Thake, personal communication), where subjects cycled in normoxia vs. hypoxia for 90 min ( $n = 6$ ), reduced p[NEFA] was observed in hypoxia during exercise.

#### Plasma Antioxidant Capacity (p[TAC])

This was high in all subjects at rest (mean 550 VEA equivalent units). This is generally thought to be indicative of a good level of fitness in comparison with healthy controls and some athletes. These levels did not change at altitude. However, in those individuals ( $n = 15$ ) with the highest scores for URTI, there was an unexpected increase in antioxidant capacity (mean 650 VEA equivalent units) upon return to the desert. There was a 14% higher antioxidant capacity at Dsrt1 ( $p < 0.001$ ) compared with baseline, and a close-to-significance 9% lower level at FTX1b compared with FTX1 and FTX2 ( $p < 0.1$ ).

#### Leptin

Insufficient plasma was available for some of the samples, thus complete data sets were only obtained on 24 individuals. Nevertheless, incomplete data sets for all subjects showed a similar pattern overall, of a significant decrease (56%) in plasma leptin ( $p < 0.001$ ) at the midpoint of FTX at altitude, which was maintained through the end of FTX and on return to the desert (data not shown).

### DISCUSSION

A decrease in p[Gln] and a relatively lower antioxidant capacity in marines during winter training at altitude occurred concomitantly with a high incidence of illness, particularly URTI. This supports data obtained previously at moderate altitude.<sup>22,23</sup> A trend, which approached significance ( $r = 0.622$ ,

$p < 0.1$ ), suggested that those with the most severe URTI symptoms score also had the greatest decrease in p[Gln]. This was similar to findings in Bailey et al.<sup>22</sup>

Parry-Billings<sup>24</sup> observed a marked exercise-induced decrease in p[Gln] in six U.K. marines after 29 weeks of intensive training; however, these samples were taken during the recovery period after training. Of particular importance in the present study is the fact that the decrease in p[Gln] occurred in fasting, resting, early morning samples, rather than after exercise as observed in previous studies. This indicates a cumulative effect on plasma glutamine levels, which is not restored overnight after prolonged exposure to physical and mental stress in harsh conditions. Such a decrease in p[Gln] may be a marker of decreased well-being and immunocompetence, in particular in the individual who has a poor stress tolerance. It is worth bearing in mind that glutamine is important in the synthesis of the antioxidant, glutathione.

Some studies have reported decreased plasma glutamine in either Olympic athletes or mountaineers undertaking prolonged, exhaustive exercise at moderate altitude, which were also associated with an increased incidence of illness, particularly URTI.<sup>7,22</sup> Oronasal breathing and consequent impairment of mucosal protection can occur during intense bouts of exercise, which may enhance the risk of URTI. However, it should be mentioned that Spence et al.<sup>25</sup> have demonstrated in a more recent study that not all those displaying prolonged symptoms actually have an infection. In the present study, marines who displayed the conventional symptoms were judged to have a URTI, and this was used as a surrogate measure of immunodepression.

The decrease in p[Gln] was maintained when the marines returned to their base in the desert, as was the incidence of URTI symptoms and, in some individuals, the severity. It has not previously been established whether the effect on p[Gln] is one of altitude per se or a combination of altitude and exhaustive exercise. For example, a previous study from this group observed a 25% decrease in p[Gln] in approximately 200 marathon runners after races at sea level in spring and autumn.<sup>6,7</sup> However, in the second study reported here, p[Gln] was measured in subjects at rest and exercise in hypoxic conditions. Hypoxia per se produced little or no change in p[Gln] in either rest or exercise. The stress of physical exertion may thus be more relevant to glutamine depletion than that of altitude. However, it is possible that the additional stress of hypoxia when exposed to repeated bouts of activity and limited recovery may contribute to decreased p[Gln].

With regard to the use of changes in p[Gln] as a predictor of illness, it is important to bear in mind that relative, rather than absolute measurements of p[Gln] must always be used. This is because of considerable inter- and intraindividual variation (from 400 to 900 μM), which means that blood glutamine can only be useful as an indicator if monitored on two or more occasions. For example, it is necessary to be able to compare a sample when an athlete is unwell with one taken during good health. In a group of Olympic triathletes in the

last two blood samples of the competitive season, there was a noticeable decrease in early a.m. p[Gln], which was concomitant with a subsequent, high incidence of URTI and fatigue.<sup>26</sup> It seems that p[Gln] may be a marker of an individual's potential vulnerability to opportunistic infections.

Serum IL-8 was high after FTX2.<sup>32</sup> This high level may well be linked to circulating numbers of neutrophils (which were higher at FTX2 than at other time points) and to the decrease in plasma glutamine observed. In terms of neutrophil oxidative burst, preliminary data from the pilot study suggested increased degranulation of neutrophils during FTX. This would occur in response to an immune challenge and tends to confirm studies described in the Introduction (see<sup>7</sup>). Under laboratory conditions neutrophilia and lymphopaenia have been observed at 4,000 m in resting participants.<sup>33</sup>

Recently the presence of glutaminase has been established in human neutrophils,<sup>34</sup> indicating that glutamine is potentially an important fuel for neutrophils. This finding is linked with previous observations that IL-8 is always decreased in the glutamine group vs. the placebo group.<sup>7</sup> This suggests a link between the high pIL8 and low glutamine observed in the present study. Increased utilization of glutamine by increased cell populations because of the inflammatory response, may account for its removal from circulation, thus leading to a decrease in p[Gln].

#### *Central Fatigue and Immunodepression*

Beneficial effects of BCAA supplementation upon mental performance after prolonged, exhaustive exercise have been observed<sup>14,35</sup> and upon physical performance during heat stress<sup>36</sup> or prolonged, exhaustive exercise.<sup>37</sup> BCAAs also act as precursors for glutamine. Triathletes who received BCAA daily for 1 month maintained p[Gln] and also had significantly fewer URTIs than those on placebo;<sup>38</sup> more recently, Hiscock et al.<sup>35</sup> observed a similar effect in marathon runners.

Tryptophan, a precursor of serotonin, competes with NEFA to bind to albumin in the blood, and with BCAA to cross the blood-brain barrier. However, no change in BCAA was observed in the present study, whereas tryptophan was decreased at all time points while the marines were training at altitude. It is reasonable to speculate that the cold during the sojourn at altitude (subjects slept in tents in the snow for 2 weeks during intense training) might have affected lipolysis, and thus the level of unbound tryptophan. Upon return to the desert, there was a marked increase in plasma NEFA and tryptophan.

The availability of plasma tryptophan is linked with immunodepression, via IFN $\gamma$  induction of indolamine<sup>2,3</sup>-dioxygenase (IDO) a rate-limiting enzyme for tryptophan metabolism. The decrease in p[FT] observed during winter field training may be associated with increased cellular immune activation, inducing the enzyme IDO to catabolize tryptophan. IDO also has antioxidant properties because of its use of the superoxide anion (produced in greater quantity at altitude) as an oxygen source.<sup>39</sup>

With regard to the increase in antioxidant capacity observed 1 month after return to the desert, it is possible that this is a response to combat the residual effect of URTI. The high incidence of URTI symptoms was still noticeable at 37 days post-deployment to altitude.

The p[NEFA] data were unusual compared with other endurance exercise studies from this laboratory, not only because there was no increase at the end of FTX but because there was a marked increase upon return to the desert. The high temperatures may have elevated lipolysis via increased blood flow and peripheral vasodilatation. Interestingly, reduced p[NEFA] was observed in hypoxia compared with normoxia during exercise in the second study. Another possibility is that these results could be explained by considering the leptin findings. A recent study (A. Taylor and L. Castell, unpublished observations) on health and illness in a range of body mass indices (BMIs) showed an apparent correlation between URTI and plasma leptin. Leptin sustains lean tissue and promotes the loss of adipose tissue through increased lipolysis. Thus, the observed reduction in plasma leptin might have had metabolic consequences, i.e., a reduction in lipolysis and reduced NEFA. There were body composition changes consistent with this idea—see Bardwell et al.<sup>40</sup> This result is also consistent with the tryptophan results; the reduction in plasma NEFA would increase the availability of binding sites on albumin, thus leading to more binding of tryptophan to plasma albumin and to reduced plasma tryptophan.

Diet is an important aspect in relation to fatty acids. The meals-ready-to-eat (MRE) at MWTC contained 36% fat, 50% carbohydrates, and 14% protein. It is unlikely that much more fat would be consumed upon return to the desert, where normal meals were available. In retrospect, it would have been useful to obtain dietary information from these individuals but this was logistically difficult, since the participants had scant time to provide the information already requested. Standard meals-ready-to-eat were provided and, when conditions permitted, one hot meal per day was delivered to the field from base camp.

#### **CONCLUSIONS**

Resting, fasting samples taken from marines in the desert and at altitude demonstrated a decrease in p[Gln] after 1 month's winter training at altitude; this low level was maintained when participants returned to the desert (at 37 days but not at 98 days). Plasma [FT], decreased upon arrival at base camp vs. desert baseline samples, remained low throughout winter training, and was restored to baseline levels upon return to the desert. The reasons and implications for this are not fully understood. Plasma [Lep] started to decrease midway through FTX at altitude in a subset of participants ( $n = 24$ ); laboratory studies in hypoxic conditions have shown conflicting results for p[Lep]. An initial increase in p[NEFA] coincided with a decrease in p[FT]. There was a marked increase in URTI symptoms and severity after FTX.

The decrease in p[Gln] in early a.m., resting samples, suggests a cumulative effect of prolonged, exhaustive exercise and psychological stress on glutamine levels in the blood. It would be interesting to investigate a possible link between p[Gln] and leptin, which has recently been suggested as a potential marker of overtraining (M. Lehmann, personal communication), and which may have a link with the incidence of URTI. The routine provision of exogenous glutamine, or a glutamine precursor, might help to combat the high incidence of URTI symptoms during intensive exercise in these extreme conditions. The correlation between URTI symptom severity and low p[Gln] suggests that p[Gln] may be a marker of an individual's potential vulnerability to opportunistic infections. A similar situation was seen in an earlier study at moderate altitude with elite athletes in training camp. Thus, a decrease in p[Gln] may be a marker of decreased well-being and immunocompetence, in particular in the individual who has a poor stress tolerance.

# ACKNOWLEDGMENTS

We thank the marines for their cheerful participation, the corpsmen who dealt with the questionnaires, and Dr. Jan Knight for expert help with antioxidant capacity measurements. Dr. Don Roberts and Stuart Jefford helped with samples; Dr. Dorothy Hudig kindly provided laboratory facilities. L.M.C. is grateful to the Office for Naval Research (ONR) for funds; W.E. was supported by the ONR Stress Physiology Program (Code 342).

# REFERENCES

1. Kramer TR, Moore RJ, Shippee RL, et al: Effects of food restrictions in military training on T-lymphocyte responses. *Int J Sports Med* 1997; 18(Suppl 1): S84-90.
2. Whitham M, Laing SJ, Dorrington M, et al: The influence of an arduous military training program on immune function and upper respiratory tract infection incidence. *Mil Med* 2006; 171: 703-9.
3. Leaf DA, Kleinman MT, Hamilton M, Barstow TJ: The effect of exercise intensity on lipid peroxidation. *Med Sci Sports Exerc* 1997; 29: 1036-9.
4. Newsholme EA, Crabtree B, Ardawi M: Glutamine metabolism in lymphocytes. Its biochemical, physiological and clinical importance. *Quart Exp Physiol* 1985; 70: 473-89.
5. Parry-Billings M, Calder P, Evans J, Newsholme EA: Does glutamine contribute to immunosuppression after major burns? *Lancet* 1990; 336: 523-5.
6. Castell LM, Poortmans J, Newsholme EA: Does glutamine have a role in reducing infections in athletes? *Eur J Appl Physiol* 1996; 73: 488-91.
7. Castell LM: Glutamine supplementation in vitro and in vivo, in exercise and immunodepression. *Sports Med* 2003; 33: 323-45.
8. Parry-Billings M, Budgett R, Koutedakis Y, et al: Plasma amino acid concentrations in the overtraining syndrome: possible effect on the immune system. *Med Sci Sports Exerc* 1992; 24: 1353-8.
9. Kingsbury KJ, Kay L, Hjelm M: Contrasting plasma free amino acid patterns in elite athletes: association with fatigue and infection. *Br J Sports Med* 1998; 32: 25-32.
10. Peters EM, Bateman ED: Ultramarathon running and upper respiratory tract infections. An epidemiological survey. *S Afr Med J* 1983; 64: 582-4.
11. Nieman DC: Immune response to heavy exertion. *J Appl Physiol* 1997; 82: 1385-94.
12. Newsholme EA, Castell LM: Amino acids, fatigue and immunodepression in exercise in *Nutrition in Sport*, pp 153-170. Oxford, Blackwell Science. RJ Maughan, 2000.
13. Acworth T, Nicholass J, Morgan B, Newsholme EA: Effects of sustained exercise on concentrations of plasma aromatic and branched-chain amino acids and brain amines. *Biochem Biophys Res Commun* 1986; 137: 149-53.
14. Blomstrand E, Hassmen P, Ek S, Ekblom B, Newsholme EA: Influence of ingesting a solution of branched-chain amino acids on perceived exertion during exercise. *Acta Physiol Scand* 1997; 159: 41-9.
15. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; 372: 425-32.
16. Matarese G, Moschos S, Mantzoros CS: Leptin in immunology. *J Immunol* 2005; 174: 3137-42.
17. Caldefie-Chezet F, Poulin A, Vasson MP: Leptin regulates functional capacities of polymorphonuclear neutrophils. *Free Radic Res* 2003; 37: 809-14.
18. Spiegel K, Tasali E, Penev P, Van Cauter E: Brief communication: sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. *Ann Intern Med* 2004; 141: 846-50.
19. Castell LM, Barton E, Hickey P, et al: Sleep deprivation, fatigue and immunodepression in humans. *Int Soc Ex Immunol*, 6th International Conference, Copenhagen, Denmark, 2003.
20. Windmueller HG, Spaeth AE: Uptake and metabolism of plasma glutamine by the small intestine. *J Biol Chem* 1974; 249: 5070-9.
21. Livesey G, Lund P: Enzymic determination of branched-chain amino acids and 2-oxoacids in rat tissues. *Biochem J* 1980; 188: 705-6.
22. Bailey DM, Castell LM, Newsholme EA, Davies B: Modulatory role of exposure time to environmental hypoxia during physical exercise: implications for glutamine metabolism and exercise performance. *Br J Sports Med* 2000; 34: 210-2.
23. Schmidt MC, Askew EW, Roberts DE, Prior RL, Ensign WY, Hesslink RE: Oxidative stress in humans training in a cold, moderate altitude environment and their response to a phytochemical antioxidant supplement. *Wilderness Environ Med* 2002; 13: 94-105.
24. Parry-Billings M: Studies of glutamine release from skeletal muscle. University of Oxford, DPhil Thesis, Oxford, U.K., 1989.
25. Spence L, Brown WJ, Pyne DB, et al: Incidence, etiology and symptomatology of upper respiratory illness in elite athletes. *Med Sci Sports Exerc* 2007; 39: 577-86.
26. Dimitriou L, Jaques R, Maw G, Whyte G, Castell LM: A longitudinal study on some potential markers of incidence of illness and immunodepression in triathletes. *Proceedings 6th International Conference, Copenhagen, International Society Exercise Immunology*, 2003.
27. Robson PJ, Blannin AK, Walsh NP, Castell LM, Gleeson M: Effects of exercise intensity, duration and recovery on *in vitro* neutrophil function in male athletes. *Int J Sports Med* 1999; 20: 128-35.
28. Castell LM, Atchley D, Bravo N, Niemeyer D, Reyes A, Bradshaw P: Effect of eight weeks of training and exhaustive exercise on some aspects of the immune system. *Int J Sports Med* 1999; 21(Suppl 1): Abstr S85.
29. Castell LM, Newsholme EA: The effects of oral glutamine supplementation upon athletes after prolonged, exhaustive exercise. *Nutrition* 1997; 13: 738-42.
30. Krzykowski K, Petersen EW, Ostrowski K, Kristensen JH, Boza J, Pedersen BK: Effect of glutamine supplementation on exercise-induced changes in lymphocyte function. *Am J Physiol* 2001; 281: C1259-65.
31. O'Riordan MG, De Beaux A, Fearon K: Effect of glutamine on immune function in the surgical patient. *Nutrition* 1996; 12: S82-4.
32. Ensign W, Castell LM: Intensive training in harsh environments. *Aviat Space Environ Med* 2004; 75: No. 4 Suppl II Abstr 220.
33. Thake CD, Mian T, Garnham AW, Mian R: Human blood leukocyte counts and neutrophil activity during acute hypocapnic hypoxia. *Aviat Space Environ Med* 2004; 75: 811-7.
34. Castell LM, Vance C, Abbott R, Marquez J, Eggleton P: Granule localization of glutaminase in human neutrophils and the consequence of glutamine utilization for neutrophil activity. *J Biol Chem* 2004; 279: 13305-10.

35. Hiscock N, Crawford R, Castell LM: Supplementation of branched chain amino acids (BCAA) in marathon runners for one month prior to competition. *Med Sci Sports Ex* 2001; 33:(Suppl I)SEI, Abst 15.
36. Mittelman KD, Ricci MR, Bailey SP: Branched-chain amino acids prolong exercise during heat stress in men and women. *Med Sci Sports Exerc* 1998; 30: 83–91.
37. Blomstrand E: A role for branched-chain amino acids in reducing central fatigue. *J Nutr* 2006; 136: S544–7.
38. Bassit RA, Sawada LA, Bacurau RFP, Navarro F, Costa Rosa LFBP: The effect of BCAA supplementation upon the immune response of triathletes. *Med Sci Sports Exerc* 2000; 32: 1214–9.
39. Sun Y: Indoleamine 2,3-dioxygenase—a new antioxidant enzyme. *Mater Med Pol* 1989; 21: 244–50.
40. Bardwell WA, Ensign WY, Mills PJ: Negative mood endures after completion of high-altitude military training. *Ann Behav Med* 2005; 29: 64–9.
41. Noakes T: *The Lore of Running*. Oxford, U.K., Oxford University Press, 1985.
42. Kemeny ME, Schedlowski M: Understanding the interaction between psychosocial stress and immune-related diseases. *Brain Behav Immun* 2007; 21: 1009–18.
43. Nourooz-Zadeh J, Ziegler D, Sohr C, Betteridge DJ, Knight J, Hotherhall J: The use of pholasin as a probe for the determination of plasma total antioxidant capacity. *Clin Biochem* 2006; 39: 55–61.

Copyright of Military Medicine is the property of Association of Military Surgeons of the United States and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.